

RAW DATA

Distillation column

working volume	50% full				
volume was calculated based on the dimintions from P&ID (pipeline & instrumentation diagram)					
working volume	245.07 gal				
Feed	#	Temp. °F	Temp. °C	Flowrate (ft3/hr)	Flowrate (g/min)
Out	25	189	87.2	204.7	25.5
Average	26	228	108.9	196.4	24.5
			98.1	200.6	25.0
Residence time	9.8	min			

Experimental plan for yeast killing

Trial#	1	2	3	4	5	6	7 (control)
Temp. °C	80	90	95	80	90	95	n/a
Time (min)	5	5	5	9	9	9	n/a
At each condition: do triplate for counting							
total plate	21						

Yeast loads

g/L	OD600
1.3	2.6

Make yeast suspension

OD600	Dilution	Actual OD600
0.5809	50	29.045

Target OD600	mL	mL	mL
2.6	Total volume	Suspension Volume	R/O water
	100	9.0	91.0

OD600	CFU	0.1mL	Dilution	Expected CFU
1	2.00E+07	2.00E+06	10000	2.00E+02

Dilution for counting

Dilution factor	1	2	3	4	5	6	7	
	10	10	10	10	10	10	10	no inoculation
	10	10	10	10	10	10	10	negativ control
	1	1	1	1	1	1	1	10000
	1	1	1	1	1	1	1	10000
	1	1	1	1	1	1	1	10000
	1	1	1	1	1	1	1	n/a
	1	1	1	1	1	1	1	n/a

Labeling	1	2	3	4	5	6	7	
	1.A	2.A	3.A	4.A	5.A	6.A	7.A	no inoculation
	1.B	2.B	3.B	4.B	5.B	6.B	7.B	negativ control
	1.C	2.C	3.C	4.C	5.C	6.C	7.C	
	1.D	2.D	3.D	4.D	5.D	6.D	7.D	
	1.E	2.E	3.E	4.E	5.E	6.E	7.E	
								n/a
								n/a

Counted colony #	1	2	3	4	5	6	7	
	0	0	0	0	0	0	218	no inoculation
	0	0	0	0	0	0	222	negativ control
	0	0	0	0	0	0	209	
	0	0	0	0	0	0	n/a	
	0	0	0	0	0	0	n/a	
	0	0	0	0	0	0	216	

Average

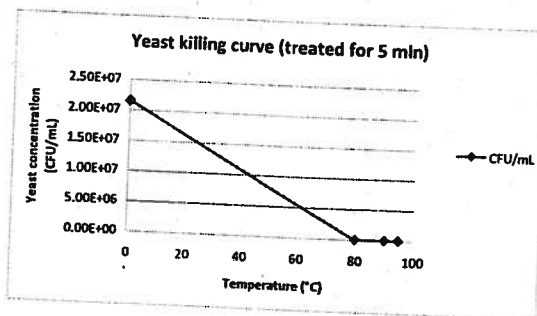
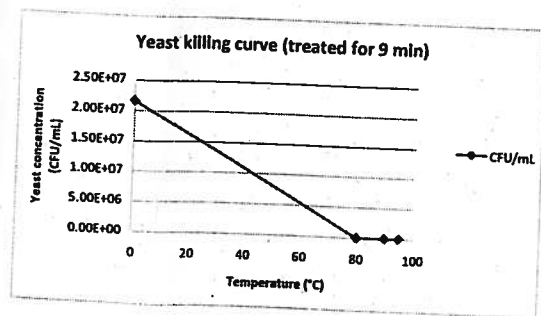
Initial yeast CFU/mL						
2.2E+07						
	1 (80°C/5min)	2 (90°C/5min)	3 (95°C/5min)	4 (80°C/9min)	5 (90°C/9min)	6 (95°C/9min)
CFU/mL	0	0	0	0	0	0
Temperature (°C)						

Treated for 5 min

Temperature (°C)	CFU/mL
0	2.16E+07
80	0
90	0
95	0

Treated for 9 min

Temperature (°C)	CFU/mL
0	2.16E+07
80	0
90	0
95	0



SOP_Ferm 021: Yeast counting on agar plate

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Author Qin Zhang

1. New work instructions

Instructions for yeast counting on YPD agar plate

2. Reference

n/a

3. Application:

This SOP is for the yeast counting on typical sterile YPD agar plate

4. Chemicals

Chemical	Specs	Safety
YPD agar plate	Sterile typical YPD agar plate	n/a
R/O water	Sterile R/O water	n/a
Aerobically cultured yeast cream	Cream from Lallemand Ethanol Technology (RN1016)	n/a

5. Equipment

Equipment
Laminar hood
5mL and 1mL Pipette
Sterile glass spreader/beads
Flame
Incubator

6. Safety

GMO yeast is used in this experiment. Please put bleach to the broth to kill the yeast before dumping them to the sink.

7. Procedure

- 7.1 Turn on the laminar hood at least 15min before using it and spray some 70% propanol on the working surface.
- 7.2 Clean out the space which is going to be used. Do not block the air flow.
- 7.3 Use the sterile flasks, pipette tips and graduated cylinder for the solution transferring.
- 7.4 Autoclave all of the solutions which are going to be used.
- 7.5 Use the typical sterile YPD agar plate for yeast colony growing.
- 7.6 Do proper dilution of the yeast suspension for counting purpose. The yeast colony number needs to be in the range of 30-300 (in volume of 100 μ L).
- 7.7 Pipette 100 μ L yeast suspension on the YPD agar plate, use the sterile glass beads or spreader to distribute the suspension well on the agar surface.
- 7.8 Incubate the agar plate in an incubator at 32°C for 48 hours before counting.

SOP_Ferm 021: Yeast killing procedure for WBE

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Author

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1. New work instructions

Instructions for yeast killing procedure for WBE support

2. Reference

n/a

3. Application:

This SOP is for the yeast killing experiment to support WBE operation

4. Chemicals

Chemical	Specs	Safety
R/O water	Sterile R/O water	n/a
Aerobically cultured yeast cream	Cream from Lallemand Ethanol Technology (RN1016)	n/a

5. Equipment

Equipment
Heating plate with automatic temperature control
Thermometer
10 mL glass testing tube
5mL and 1mL Pipette
R/O water

6. Safety

GMO yeast is used in this experiment. Please put bleach to the broth to kill the yeast before dumping them to the sink.

7. Procedure

- 7.1 Turn on the heating plate and put a 1 liter beaker on the plate. The heating plate has automatic temperature control.
- 7.2 Do certain dilution for preparing the yeast suspension. The yeast concentration needs to match the yeast dosage in WBE.
- 7.3 Pipette the yeast suspension in the narrow glass testing tube and covered with parafilm to prevent water loss during killing process.
- 7.4 The tested temperatures are 80, 90 and 95 °C. The heating times are 5 and 9 min.
- 7.5 After heating, the culture is transferred to the eppendorf centrifuge tube and store on ice for cooling the solution down.
- 7.6 The yeast suspension with heat treatment will be used for yeast counting on YPD agar plate later on.
- 7.7 The yeast counting on YPD agar plate is based on SOP_Ferm 021 yeast counting on agar plate.

SOP: Aerobic propagation in shake flask

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Author Qin Zhang

1. New work instructions

Instructions for aerobic yeast propagation in shake flask.

2. Reference

n/a

3. Application:

This SOP is for the yeast propagation in the baffled shake flask.

4. Chemicals

Chemical	Specs	Safety
Xylose	Sterile 200 g/L solution	n/a
Yeast Extract	Sterile 100 g/L solution	n/a
R/O water	Sterile R/O water	n/a
Yeast cream	From Lallemand Ethanol Technology (RN1016)	n/a
Lactocide 247	From Lallemand Ethanol Technology	n/a

5. Equipment

Equipment
Shaker
500mL baffled flask
Laminar hood
100 mL Graduated cylinder
5mL and 1mL Pipette

6. Safety

GMO yeast is used in this experiment. Please put bleach to the broth to kill the yeast before dumping them to the sink.

7. Procedure

- 7.1 Turn on the laminar hood at least 15min before using it and spray some 70% propanol on the working surface.
- 7.2 Clean out the space which is going to be used. Do not block the air flow.
- 7.3 Use the sterile flasks, pipette tips and graduated cylinder for the solution transferring.
- 7.4 Autoclave all of the solutions which are going to be used.
- 7.5 The formulations of the medium for using are listed below in Table1.
- 7.6 The working volume (medium volume) of 500mL baffled flask need to be below 100mL for aerobic growth (propagation). The calculations listed in Table2 were based on final medium volume of 100mL. If less medium volume is required, the volume of each concentrated solution need to be calculated respectively.

Table 1. General propagation medium

Medium Ingredients	Concentration
Carbon source: Xylose	10g/L
Nitrogen source: Yeast extract	10g/L
Antibiotics: Lactocide 247	4ppm